UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: September 28, 2021

SUBJECT: D/L-Glufosinate Ammonium Supplemental Data Evaluation Record (DER)

PC Code: 128850
Decision No.: 563271
Petition No.: N/A
Risk Assessment Type: N/A
TXR No: 0058206
MRID Nos.: 46455701

DP Barcodes: D462903
Registration No.: 94609-R
Regulatory Action: N/A
Case No.: N/A
CAS No.: 77182-82-2
40 CFR: N/A

FROM: Sarah Dobreniecki Ph.D., Biologist
Risk Assessment Branch VII
Health Effects Division, 7509P

THROUGH: Michael Metzger, Branch Chief
Risk Assessment Branch V/VII
Health Effects Division, 7509P

TO: Heather McFarley, Risk Manager
Manjula Unnikrishnan, Risk Manager Reviewer
Fungicide and Herbicide Branch
Registration Division, 7505P

I. CONCLUSIONS

During registration of the new active ingredients, L-glufosinate ammonium and L-glufosinate acid, the D/L-glufosinate ammonium developmental neurotoxicity study was updated to reflect current practice. The conclusions of these updates are summarized below.

II. RESULTS/DISCUSSION

Nemec, M.D. (2004) A dietary developmental neurotoxicity study of glufosinate ammonium in rats. WIL Research Laboratories, Inc., Ashland, Ohio. Study Number WIL-21202; August 19, 2004. MRID 46455701. Unpublished.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46455701), glufosinate-ammonium (50.8% a.i., lot# AAKJ00053) was administered in the diet to 25 mated female Crl:CD®(SD)IGS BR rats/dose at nominal concentrations of 0, 200, 1000 and 4500 ppm[1] from gestation day (GD) 6 through lactation day (LD) 21. Average doses to the animals were 0, 14, 69 and 292 mg/kg/day during gestation and 0, 36, 176 and 756 mg/kg/day during lactation for the 0, 200, 1000 and 4500 ppm groups, respectively. A Functional Operational Battery (FOB) was performed on 25 dams/dose on GDs 6 and 13 and on LDs 10 and 21. On postnatal day (PND) 4, litters were culled to yield five males and five females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (watermaze testing), and neuropathology at study termination (PND 72). On PND 21, the whole brain was collected from 10 pups/sex/close group for micropathology examination and morphometric analysis. Pup physical development was evaluated at the time of body weight measurements. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No parental females died during the study. The only clinical sign was light-colored feces, which occurred primarily between GDs 8 and 13, in dams at 4500 ppm. No treatment-related clinical signs were observed in dams during the FOB. Beginning on GD 7 and continuing throughout gestation, mean body weight in the 4500-ppm group was significantly decreased (4-8%, relative to control). Mean body weight loss was observed in all treated groups at the beginning of treatment (GDs 6-7 and 6-9) but overall (GDs 6-20) weight gain was significantly decreased (27% of control value) only in the 4500-ppm group. A decrease (10% of control value) in overall weight gain at 1000 ppm was also observed. Mean food consumption during gestation was significantly decreased (8-17% of control value) in the 1000 and 4500 ppm groups. Mean body weight was decreased (9-10% of control value) in the 4500-ppm group through the first half of the lactation period; however, overall (LD 1-21) mean body weight gain was increased in treated groups relative to control. Mean food consumption was significantly decreased (14% of control value) throughout lactation in the 4500-ppm group. No treatment-related effects were observed in reproductive parameters or at gross necropsy.

The maternal LOAEL is 4500 ppm (292 mg/kg/day) based on decreased body weight, body weight gain, and food consumption during gestation and lactation. The maternal NOAEL is 1000 ppm (69 mg/kg/day).

No treatment-related effect on the mean number of pups born, mean live litter size or sex ratio per litter was observed. A significant treatment-related decrease in postnatal survival occurred on PNDs 0-1 and PND 0-4 in the 4500-ppm group, due mostly to total litter losses. The total number of pups found dead during the pre-weaning period was 31, 13, 17 and 60 in the control, 200, 1000 and 4500 ppm groups, respectively. Beginning on PND 4 and continuing throughout the lactation period, mean body weight was decreased in the 1000 ppm (8-11% of control value) and 4500 ppm (13-20% of control value) male and female offspring. Mean body weight gains were decreased in the 4500-ppm male (12-35% of control value) and female (9-36% of control value) offspring and in 1000 ppm males (6-23% of control value) and females (9-23% of control value). Mean post-weaning (PNDs 28-70) body weight was decreased in males (6-9% of control value) and females (7-10% of control value) at 4500 ppm. Post-weaning body weight gain was

[1] Dose levels were corrected for purity

decreased in males and females at 4500 ppm (6-9% of control value). The average onset of preputial separation in males and vaginal opening in females was not affected by treatment.

Total and ambulatory motor activity counts were increased in males and females at 1000 ppm (1.2-2.5X control value) and 4500 ppm (1.2-2.8X control value) on most of the testing days. Statistically significant increases in several 15-minute interval motor activity counts were observed on PNDs 21 and 61 in all groups of treated males and on PND 21 in females at 1000 ppm and 4500 ppm. No statistically significant differences between treated and control groups were observed in auditory startle response or learning and memory.

Brain weight measurements and gross and microscopic necropsy findings were not affected by treatment. For the PND 72 morphometric measurements, decreased mean vertical height between the layers of pyramidal neurons in the hippocampal formation in Level 3 was observed in males at 4500 ppm; the value was outside the historical control range. Females at 4500 ppm had significantly decreased (9% of control value) radial thickness of the cortex in Level 3, which was slightly outside the historical control range. A dose-dependent decrease in mean length of the ventral limb of the dentate hilus (Level 3) was observed in both males (9-15%) and females (12-20%) at 200 ppm. Statistical significance was reached in females at 200 ppm. However, this effect was not considered adverse until 1000 ppm as the percent change observed for both sexes at 200 ppm was within the percent coefficent of variation (CV) of the controls and within the historical control range. The decrease observed at the lowest dose in males and females aged 72 days is likely the result of two male animals and one female animal that fell outside of the historical control range. No evidence of a decrease in the dentate hilus was evident in the PND 21 animals at the lowest dose tested. Starting at the mid-dose in males a slight decrease was evident (↓6%) beginning on PND 21. A decrease was not present in PND 21 females at any of the tested dose levels. In addition, D/L glufosinate DNT *in vitro* studies have been conducted by the EPA's Office of Research and Development (ORD) since the initial evaluation of the morphometric data. Briefly, D/L-glufosinate ammonium was tested in a network formation assay in developing rat cortical networks and a neurite outgrowth assay in human induced pluripotent stem cell (IPS)-derived neurons. D/L-glufosinate ammonium was without effect on neurite outgrowth in human IPS-derived neurons or neural network formation in rat primary neural cultures. High-throughput toxicokinetics (HTTK) and *in vitro* to *in vivo* extrapolation (IVIVE) approaches were used to demonstrate that the highest concentration tested in the *in vitro* studies (30 μM) would translate to an administered equivalent dose (AED) of 45 mg/kg/day for rats. The lack of activity in the *in vitro* assays up to an AED of 45 mg/kg/day further supports establishing the NOAEL at 14 mg/kg/day.

The offspring LOAEL is 1000 ppm (69 mg/kg/day) based on brain morphometric changes (decrease in the mean length of the ventral limb of the dentate hilus), increased motor activity, and decreased body weight. The offspring NOAEL is 200 ppm (14 mg/kg/day).

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426).

COMPLIANCE: Signed and dated Flagging, GLP, and Data Confidentiality statements were Provided.

EPA Reviewer: Sarah Dobreniecki Ph.D. **Signature**: Sarah Dobreniecki
Risk Assessment Branch VII, Health Effects Division (7509P) **Date:** 7-14-2021

EPA Secondary Reviewer: John Liccione Ph.D. **Signature**: John Liccione
Risk Assessment Branch V, Health Effects Division (7509P) **Date:** 7-14-2021

Template version 03/12

TXR#: 0058206

DATA EVALUATION RECORD – Supplemental
See TXR 0053106 and 0057119 for previous reviews

STUDY TYPE: Developmental Neurotoxicity – Rat; OPPTS 870.6300; OECD 426

PC CODE: 128850 **DP BARCODE:** D462903

TEST MATERIAL (PURITY): Technical Grade D/L-Glufosinate Ammonium (50.8% a.i.)

SYNONYMS: 2-amino-4-(hydroxymethylphosphinyl)butanoic acid

CITATION: Nemec, M.D. (2004) A dietary developmental neurotoxicity study of glufosinate ammonium in rats. WIL Research Laboratories, Inc., Ashland, Ohio. Study Number WIL-21202; August 19, 2004. MRID 46455701. Unpublished.

SPONSOR: Bayer CropScience, Research Triangle Park, NC

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No parental females died during the study. The only clinical sign was light-colored feces, which occurred primarily between GDs 8 and 13, in dams at 4500 ppm. No treatment-related clinical signs were observed in dams during the FOB. Beginning on GD 7 and continuing throughout gestation, mean body weight in the 4500-ppm group was significantly decreased (4-8%, relative

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to control). Mean body weight loss was observed in all treated groups at the beginning of treatment (GDs 6-7 and 6-9) but overall (GDs 6-20) weight gain was significantly decreased (27% of control value) only in the 4500-ppm group. A decrease (10% of control value) in overall weight gain at 1000 ppm was also observed. Mean food consumption during gestation was significantly decreased (8-17% of control value) in the 1000 and 4500 ppm groups. Mean body weight was decreased (9-10% of control value) in the 4500-ppm group through the first half of the lactation period; however, overall (LD 1-21) mean body weight gain was increased in treated groups relative to control. Mean food consumption was significantly decreased (14% of control value) throughout lactation in the 4500-ppm group. No treatment-related effects were observed in reproductive parameters or at gross necropsy.

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Total and ambulatory motor activity counts were increased in males and females at 1000 ppm (1.2-2.5X control value) and 4500 ppm (1.2-2.8X control value) on most of the testing days. Statistically significant increases in several 15-minute interval motor activity counts were observed on PNDs 21 and 61 in all groups of treated males and on PND 21 in females at 1000 ppm and 4500 ppm. No statistically significant differences between treated and control groups were observed in auditory startle response or learning and memory.

Brain weight measurements and gross and microscopic necropsy findings were not affected by treatment. For the PND 72 morphometric measurements, decreased mean vertical height between the layers of pyramidal neurons in the hippocampal formation in Level 3 was observed in males at 4500 ppm; the value was outside the historical control range. Females at 4500 ppm had significantly decreased (9% of control value) radial thickness of the cortex in Level 3, which was slightly outside the historical control range. A dose-dependent decrease in mean length of the ventral limb of the dentate hilus (Level 3) was observed in both males (9-15%) and females (12-20%) at 200 ppm. Statistical significance was reached in females at 200 ppm. However, this effect was not considered adverse until 1000 ppm as the percent change observed for both sexes at 200 ppm was within the percent coefficent of variation (CV) of the controls and within the

historical control range. The decrease observed at the lowest dose in males and females aged 72 days is likely the result of two male animals and one female animal that fell outside of the historical control range. No evidence of a decrease in the dentate hilus was evident in the PND 21 animals at the lowest dose tested. Starting at the mid-dose in males a slight decrease was evident (↓6%) beginning on PND 21. A decrease was not present in PND 21 females at any of the tested dose levels. In addition, D/L glufosinate DNT *in vitro* studies have been conducted by the EPA's Office of Research and Development (ORD) since the initial evaluation of the morphometric data. Briefly, D/L-glufosinate ammonium was tested in a network formation assay in developing rat cortical networks and a neurite outgrowth assay in human induced pluripotent stem cell (IPS)-derived neurons. D/L-glufosinate ammonium was without effect on neurite outgrowth in human IPS-derived neurons or neural network formation in rat primary neural cultures. High-throughput toxicokinetics (HTTK) and *in vitro* to *in vivo* extrapolation (IVIVE) approaches were used to demonstrate that the highest concentration tested in the *in vitro* studies (30 μM) would translate to an administered equivalent dose (AED) of 45 mg/kg/day for rats. The lack of activity in the *in vitro* assays up to an AED of 45 mg/kg/day further supports establishing the NOAEL at 14 mg/kg/day.

The offspring LOAEL is 1000 ppm (69 mg/kg/day) based on brain morphometric changes (decrease in the mean length of the ventral limb of the dentate hilus), increased motor activity, and decreased body weight. The offspring NOAEL is 200 ppm (14 mg/kg/day).

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426).

COMPLIANCE: Signed and dated Flagging, GLP, and Data Confidentiality statements were provided.

COMMENTS: This is a revised executive summary that was prepared to update the conclusions of the previous DER. Previously, the offspring LOAEL was 200 ppm (14 mg/kg/day) based on brain morphometric changes. The offspring NOAEL was not observed. The LOAEL has been updated to 1000 ppm (69 mg/kg/day) with a NOAEL of 200 ppm (14 mg/kg/day). This decision was based on the following weight-of-the-evidence approach:

- At the lowest dose tested the percent change in the decrease of the length of the dentate hilus observed for both sexes was within the percent of coefficient of variation (CV) of the controls and within the historical control range provided in the initial DNT study report (MRID 46455701) (**Table 1**). The historical control data that was presented during the 2015 re-evaluation (MRID 49331202) was also taken into consideration; however, more weight was placed on the original historical control data set as compared to the re-evaluation historical control data. As the later was collected over a wider range of years, and a small change in methodology and/or angle of the cut may result in small variations in morphometric measurements, the original historical control data set that was conducted closer to the year of the glufosinate DNT study is more appropriate.

- A more robust decrease in the length of the dentate hilus was evident at 1000 ppm (69 mg/kg/day) with a 12% and 16% decrease in the dentate hilus of PND 72 males and females, respectively, vs. a 9% and 12% decrease at the lowest dose tested.
- The decrease observed at the lowest dose in males and females aged 72 days is likely the result of only two male animals and one female animal that fell outside of the historical control range.
- No evidence of a decrease in the length of the dentate hilus was evident in the PND 21 data at the lowest dose tested. Starting at the mid-dose in males a slight decrease was evident (↓6%) beginning on PND 21. A decrease was not observed in PND 21 females at any of the tested dose levels.
  The re-evaluation of morphometric data that was conducted in 2015 was also considered (see TXR# 0057119, J. Liccione, 02-FEB-2015 for Agency conclusions). The CVs from the study were fairly tight and there was not a substantial amount of variability in the measurements. The variability may be an artifact of slight biological changes in the dentate hilus and/or slight changes in the methodology that was used to prepare the sections. Overall, when the initial evaluation, and the re-evaluation data were examined together, the percent change was not grossly different (**Table 2**). The most significant change was noted in the control females re-evaluation which changed the percent decrease across all doses tested.
- Since the 2015 re-evaluation conducted by the Agency, DNT *in vitro* data has been submitted and evaluated. This data supports increasing the LOAEL to 1000 ppm (69 mg/kg/day). Briefly, D/L-glufosinate ammonium was tested in a network formation assay in developing rat cortical networks and a neurite outgrowth assay in human induced pluripotent stem cell (IPS)-derived neurons. D/L-glufosinate ammonium was without effect on neurite outgrowth in human IPS-derived neurons or neural network formation in rat primary neural cultures. High-throughput toxicokinetics (HTTK) and *in vitro* to *in vivo* extrapolation (IVIVE) approaches were used to demonstrate that the highest concentration tested in the *in vitro* studies (30 µM) would translate to an administered equivalent dose (AED) of 45 mg/kg/day for rats. The lack of activity in the *in vitro* assays up to an AED of 45 mg/kg/day further supports establishing the NOAEL at 14 mg/kg/day.
- During the 2015 re-evaluation of the brain morphometric data, uncertainties in the data were acknowledged including the poor quality of the sections, the lack of reliable and accurate brain morphometric measurements, the exclusion of measurements due to poor section quality (which was also acknowledged by the Registrant), the lack of laboratory experience with conducting morphometric measurements of the dentate hilus, and the restricted comparison of the group mean for the control animals to the group means of the historical control studies which is limited by the refinement of the cursor placement. Since the 2015 re-evaluation, the DNT *in vitro* data (discussed above) has been evaluated by the Agency and provides additional evidence to support a lack of adverse treatment related effects up to an AED of 45 mg/kg/day for rats. This further supports establishing the NOAEL at 14 mg/kg/day.

Table 1: Mean (±SD) Brain Morphometric Measurements (cm)[a]

Dietary Concentration					
Parameter	**0**	**200 ppm 14 mg/kg/day**	**1000 ppm 69 mg/kg/day**	**4500 ppm 292 mg/kg/day**	**Historical Control Range (cm)[c]**
Males – Day 21					
Length ventral limb dentate hilus	0.124 ± 0.016 CV= 13%	0.123 ± 0.009 CV= 7%	0.117 ± 0.022 (↓6%) CV= 19%	0.113 ± 0.010 (↓9%) CV= 9%	0.117-0.122
Males – Day 72					
Length ventral limb dentate hilus	0.154 ± 0.022 CV= 14%	0.140 ± 0.014 (↓9%) CV= 10%	0.136 ± 0.016 (↓12%) CV= 12%	0.131 ± 0.017* (↓15%) CV= 13%	0.136-0.164 (0.126-0.154)[d]
Females – Day 21					
Length ventral limb dentate hilus	0.108 ± 0.009 CV= 8%	0.120 ± 0.005 CV= 4%	0.118 ± 0.018 CV= 15%	0.114 ± 0.015 CV= 13%	0.117-0.121
Females – Day 72[b]					
Length ventral limb dentate hilus	0.149 ± 0.018 CV= 12%	0.131 ± 0.009* (↓12%) CV= 7%	0.125 ± 0.015** (↓16%) CV= 12%	0.119 ± 0.011** (↓20%) CV= 9%	0.127-0.152 (0.127-0.149)[d]

[a] data obtained from pages 299-300, 303-304, 312-313, 326-327 in the study report
[b] data from one female in the 4500-ppm group that had dilation of the lateral ventricles of the cerebral cortex and the third ventricle of the hypothalamus on PND 72 was not included in the calculation of the mean for that group
[c] the study dates (i.e. the year(s) the studies were conducted) for historical control data are not available in the study report. Group mean values from 3 DNT studies.
[d] historical control range from Bayer's re-evaluation. Group mean values from 12 DNT studies.
N = 9-10
CV = coefficent of variation
* Statistically significant different from the control, $p<0.05$
** Statistically significant different from the control, $p<0.01$
Number in parentheses is the % decrease, relative to control, calculated by the reviewer

Table 2: Comparison of Mean (±SD) Brain Morphometric Measurements (cm)[a] Across the Initial Evaluation and the 2015 Re-evaluation

Dietary Concentration				
Parameter	**0**	**200 ppm 14 mg/kg/day**	**1000 ppm 69 mg/kg/day**	**4500 ppm 292 mg/kg/day**
Initial Evaluation Males – Day 72				
Length ventral limb dentate hilus	0.154 ± 0.022 CV=14%	0.140 ± 0.014 (↓9%) CV=10%	0.136 ± 0.016 (↓12%) CV=12%	0.131 ± 0.017* (↓15%) CV=13%
Initial Evaluation Females – Day 72				
Length ventral limb dentate hilus	0.149 ± 0.018 CV= 12%	0.131 ± 0.009* (↓12%) CV=7%	0.125 ± 0.015** (↓16%) CV=12%	0.119 ± 0.011** (↓20%) CV=9%
Re-evaluation Males – Day 72				
Length ventral limb dentate hilus	0.151 ± 0.019 CV=13%	0.135 ± 0.013 (↓11%) CV=10%	0.133 ± 0.016 (↓12%) CV=12%	0.127 ± 0.014 (↓16%) CV=11%
Re-evaluation Females – Day 72				
Length ventral limb dentate hilus	0.135 ± 0.022 CV=16%	0.129 ± 0.014 (↓4%) CV=11%	0.125 ± 0.016 (↓7%) CV=13%	0.116 ± 0.007 (↓14%) CV=6%